



Safety of AQUI-S®20E (10% Eugenol) as a Sedative for Yellow Perch

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Sedatives are physical or chemical agents that initially induce a calming effect on vertebrate animals, and subsequently induce loss of equilibrium, mobility, consciousness, and reflex action. Fisheries professionals routinely sedate fish for a variety of purposes including collection of tissue samples or morphometric data, implantation of tags or tracking devices, spawning, and transport. Sedating fish before handling can minimize stress and physical injury to the fish and also help protect the handler. Ideally, a fish sedative is safe, effective, easy to administer, predictable, and inexpensive. Also, it is desirable that the sedative have no mandated withdrawal period so that treated fish can be returned to, or released into public waters immediately after treatment.

Currently, only TRICAINES is approved by the U.S. Food and Drug Administration (FDA) for the temporary immobilization of fish and other aquatic, cold-blooded animals. This drug is an effective sedative and widely used by fisheries professionals; however, a 21-d withdrawal period is required after use before treated fish may enter the human food chain through stocking or release. For many field applications, holding fish for 21 d post-sedation is not practical and seriously compromises management or research activities.

Efforts are underway to generate data to support FDA approval of AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) as an immediate-release fish sedative.

Bowker et al. (2013) demonstrated that 40 mg/L eugenol consistently sedated a variety of coolwater finfish to handleable within 2 min at a mean water temperature of 18°C, and this dose will be proposed as the *lowest* efficacious dose. A fish was determined to be handleable when it lost equilibrium and the ability to swim, could easily be caught by hand, and did not struggle while being measured for length. However, FDA approval will also require demonstrating that fish can be safely exposed to (a) the proposed *highest* efficacious dose (80 mg/L eugenol) (b) a dose 50 % greater than that (120 mg/L eugenol), and (c) for durations exceeding those necessary to sedate these fish to handleable. As such, we conducted a study to determine whether there is an adequate margin of safety associated with exposing fingerling Yellow Perch (YEP) *Perca flavescens* (a representative coolwater finfish) to 0, 80, or 120 mg/L eugenol. An adequate margin of

safety was defined as an exposure dose and duration in which test fish survival was $\geq 95\%$ when exposed for 3-4 min longer than the ET80^a (effective time for 80% of the fish to become sedated) for the highest efficacious dose and 2-3 min longer than the ET80 for the dose 50% greater than that.

Methods

The study was conducted at the Bozeman Fish Technology Center, Bozeman, Montana in August 2012. Fingerling YEP were exposed to AQUI-S®20E at doses of 0, 80, or 120 mg/L eugenol. Mean total length and weight of 20 fish sampled from the reference population 2 d before the start of the study for fish health evaluation were 6.1 cm (SD, 1.0) and 2.7 g (SD, 1.4).

One day before the start of the study, times to individually sedate 15 fish to handleable were measured for each dose and the ET80 for each dose was calculated. Four exposure durations were selected for each exposure dose such that T1 and T2 exposure durations yielded survival data in the range of 95 - 100%; T3 yielded survival data in the 70 - 90% range; and T4 yielded survival data in the 50 - 70% range. The four exposure durations assigned to 0 mg/L were identical to those assigned to 80 mg/L, which ensured that groups of control fish were tested at the longest set of exposure durations used in the study. Hence, there were 12 exposure regimen combinations (3 doses \times 4 exposure durations per dose).

Testing consisted of exposing three replicate groups (n = 15 fish per group) of test fish to each of the 12 exposure regimens, and each exposure event was followed by a 24-h recovery period. Fish were sedated in 3.8 L plastic buckets under static-bath conditions for predetermined durations, and allowed to recover in 111-L fiberglass tanks plumbed with flowing water. Water temperature and dissolved oxygen (DO) concentration were measured in each exposure container before placing fish in the solution. Sedative solution samples were collected from all exposure containers and analyzed to verify eugenol concentrations by UV-Vis spectrophotometry. Fish-response data included survival, general fish behavior during sedation and recovery, and fish health recorded for dead fish collected within 30 min of transfer to recovery tanks and subsamples of live fish collected from each tank 24-h post-exposure. All fish were

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examined visually during gross necropsy.

Results and Discussion

All fish exposed to 0 mg/L eugenol survived. At 80 mg/L eugenol, acceptable survival ($\geq 95\%$) was observed among fish exposed for 10.50 min (T4; 9.6 min beyond the ET80 for this dose; Table 1). Based on these results, the margin of safety extended to at least 10.5 min and the safety break point was not identified because we didn't expose fish for a duration that resulted in survival $< 95\%$. At 120 mg/L eugenol, acceptable survival was observed among fish exposed for 6.00 min (T3; 5.2 min beyond the ET80 for this dose) but decreased to an unacceptable level when exposed for 6.75 min (T4; 6.0 min beyond the ET80; Table 1). Based on these results, the margin of safety extended to at least 6.00 min and the safety break point was between 6.00 and 6.75 min.

Gross examination of external and internal tissues of all fish sampled appeared normal regardless of exposure dose or duration and regardless of whether a fish was alive or dead when collected.

Temporary "head-shaking" and "coughing" behavior was observed in $\geq 75\%$ of fish upon immersion in AQUI-S®20E, regardless of exposure dose or duration. This behavior lasted no more than 10 – 30 sec. Fish that recovered from sedation resumed normal behavior.

Mean eugenol concentrations from the 80 and 120 mg/L eugenol exposure buckets were 80.9 (SD, 2.0) and 122.9 (SD, 2.4) mg/L eugenol. No eugenol was detected in samples collected from the

0 mg/L exposure group.

Mean water temperatures in exposure buckets and recovery tanks was 18.8°C (SD, 0.3) and 19.6°C (SD, 0.1). Mean DO concentrations in exposure buckets before and after fish were sedated were 7.0 (SD, 0.2) and 6.8 (SD, 0.3) mg/L. Mean DO concentrations in recovery tanks at the beginning and end of the 24-h recovery period were 6.8 (SD, 0.2) and 6.9 (SD, 0.5) mg/L. Water alkalinity and hardness were 169 mg/L and 216 mg/L (both measured as CaCO₃); and pH was 7.8. Mean pH measurements in AQUI-S20E bulk working solutions for the 0, 80 and 120 mg/L eugenol batches were 8.3, 8.2, and 8.1.

Based on survival, there was an adequate margin of safety associated with overexposing fingerling YEP to 80 or 120 mg/L eugenol. No gross lesions were detected that were considered to be of biological concern that might have indicated potential toxicity to the test fish. Results from this study were submitted to FDA to support a claim that AQUI-S20E is safe to freshwater finfish.

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References

Bowker, J.D., D. Carty, and N. Wandelaar. Use of AQUI-S®20E and BENZOAK® to Sedate Walleye, Yellow Perch, Common Carp, and Fathead Minnow to Handleable. U.S. Fish and Wildlife Service Aquatic Animal Drug Approval Partnership Program Drug Research Information Bulletin #31.

Table 1. Relative survival of fingerling Yellow Perch exposed to 80 or 120 mg/L eugenol for various durations. Acceptable survival was $\geq 95\%$.

Eugenol Dose	ET80 (min)	Exposure Duration (min)			
		T1	T2	T3	T4
80	0.90	5.0	7.0	9.0	10.5
		100%	100%	100%	96%
120	0.78	4.0	5.0	6.0	6.75
		100%	100%	100%	93%